INFRARED STUDIES OF HISTAMINE, HISTIDINE,
AND SEVERAL ANTIHISTAMINES.

by

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INTRODUCTION

A great amount of interest has developed in recent years regarding histamine and the role it plays in allergy. Studies of this problem have been confined chiefly to the fields of physiology and pharmacology. With the introduction of the so-called antihistamines it is becoming more and more apparent that an understanding of the chemistry involved in the allergic reaction would aid materially in the development of antiallergic drugs.

Histamine is the name commonly applied to the compound β-(4-imidazole)ethylamine. It is generally believed to be present in the body as a result of the degradation of the essential amino acid histidine. The exact mechanism whereby histidine is converted to histamine is not understood. This will be discussed more fully in a later paragraph.

Histamine was first synthesized in the laboratory by Windaus and Vogt (18) in 1907. These workers were not aware of the physiological properties possessed by the compound, and it was not until Barger and Dale (3) isolated histamine from certain ergot extracts in 1910 that the action of the compound upon various tissues of the body became known.

Investigations of the properties of histamine since the discoveries of Barger and Dale have been concerned mainly with its physiological actions. According to Carver (14) parenterally administered histamine shows three main physiological actions: (1) a stimulating action upon smooth muscle; (2) a dilating action upon capillaries and arterioles with constriction of the remainder

of the vascular system; and, (3) a stimulating action upon certain glands. When applied to the skin histamine causes local inflammation and the appearance of wheals.

Similarities between the effects produced by the administration of histamine and those appearing in anaphylaxis and allergic reactions have been noted by many investigators (14). Much data has appeared in the literature indicating that the appearance of allergic signs coincides with an increase in the amount of histamine in the blood and sensitive tissues. As a result of these observations several theories have arisen concerning the relation of histamine to the allergic manifestations. The most widely accepted explanation of the allergic reaction regards it as being due to the union of antigen and antibody either in the blood stream or in other tissue, followed by a liberation of histamine and other tissue metabolites. The ensuing effects can thus be attributed to these liberated substances.

The origin of histamine in the tissues of the body is not understood. As mentioned previously histamine is considered by some investigators to be the degradation product of histidine. It is thought that the decarboxylation of this amino acid is brought about by the enzyme histidine decarboxylase. Thus,

$$N - C - C - C - OH \quad \xrightarrow{-CO_2} \quad N - C - C - C - NH_2$$

Histidine   Histamine
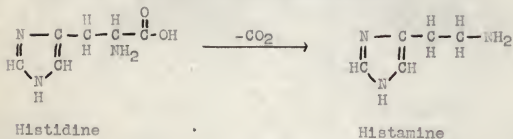
Ackermann (2), in 1910, first demonstrated that the bacterial de-

composition of histidine produced a substance markedly similar
to histamine in physiological properties. Holtz (9) found that
irradiation of aqueous histidine solutions with ultravoilet light
in the absence of oxygen resulted in the formation of histamine
in small yields. Ellinger (6) has stated that irradiation of
even dry histidine results in histamine formation. Holtz and
Heise (11) have reported that histidine is partially converted to
histamine by the action of ascorbic acid or glutathione in the
presence of oxygen. The remainder of the histidine was found to
be deaminated. This work has been questioned by Abderhalden (1)
who found that in the presence of ascorbic acid nearly all the
amino nitrogen was converted to ammonia. The findings of Ellinger
are also in doubt since it has been suggested (9) that either ir-
radiation by ultravoilet light or intensive heating of crystalline
histidine results in deamination rather than decarboxylation. Ac-
cording to von Richter (17) histamine is produced when histidine
is heated in strong mineral acid solution.

It has been shown that histamine is removed rapidly from the
blood following intravenous injection (14). It is believed that
the histamine is quickly taken up by the blood cells and rendered
inactive. The histamine may be quantitatively recovered. This
fact seems to indicate that it is capable of combining, by means
of a weak chemical bond, with the proteins of the blood cells.
The liberation of histamine by the action of proteolytic enzymes
is further evidence for such bond formation.

In 1937 Bovet (14) discovered that certain ethylenediamines
and aminoethyl ethers were powerful antagonists toward histamine.

Since that time the list of compounds exhibiting histamine antag-
onism to some degree has grown to tremendous proportions. All,
however, have certain structural similarities. They may be re-
presented by the general formula

$$R-X-\overset{\displaystyle H}{\underset{\displaystyle H}{C}}-\overset{\displaystyle H}{\underset{\displaystyle H}{C}}-N\big<$$
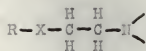
where R is an aromatic nucleus and X may be oxygen, nitrogen, or
carbon. The series of compounds in which X is nitrogen has been
found to possess a higher degree of antagonism toward histamine
than either of the other series.

Correlation of the molecular structure of various compounds
with their antihistaminic activity has led to several general-
izations (19). For a compound in the ethylene diamine series to
show antihistaminic activity both nitrogens must be tertiary.
Maximum activity is obtained when the side chain is the dimethyl-
aminoethyl group. Higher alkyl substituents on the amine group
leads to inactive compounds. An increase in the side chain length
from ethylene to trimethylene produces a sharp drop in activity.

It has been suggested that antihistaminic compounds have
some special affinity for certain components of effector cells
(14). The antagonism toward histamine might be thought of as a
competition between the two molecules for a particular site upon
the cell. In view of the above mentioned generalizations regard-
molecular structure, it might be that certain physical properties
of the antihistamine, such as molecular diameter and molecular
weight, play a dominant role in determining the degree of activ-
ity possessed by that compound.

The methods of infrared spectroscopy have been employed in the solution of many problems concerning molecular structure. It was thought that some of the problems pertaining to the origin of histamine in the body and its relation to the proteins of the blood might successfully be examined by the use of infrared spectroscopy.

The results of this investigation are divided into three sections: (1) a preliminary study of the spectra of three antihistamines; (2) a comparison of the spectra of histidine and histamine, with an examination of the spectrum of the thermal decomposition product of histidine; and, (3) the changes produced in the spectrum of bovine serum albumin as a result of interaction with histamine.

INFRARED SPECTROSCOPY

In recent years infrared spectroscopy has found wide application as a tool in chemical research. A voluminous literature has accumulated, particularly in the field of molecular structure studies. A thorough survey of the literature up to 1943 is given by Barnes et al. (4).

The usefulness of infrared spectroscopy lies in the fact that nearly all organic compounds show selective absorption of radiation of frequencies corresponding to the infrared region of the electromagnetic spectrum. This region, expressed in wavelength units, extends from 1 to 350 microns. The atoms of any molecule not at absolute zero are oscillating about their equilibrium positions.

The frequencies of such oscillations are functions of the atomic masses, the strength of the interatomic bonds, and the geometric configuration of the molecule. If such oscillations are considered to be simple harmonic in that the restoring force is proportional to the displacement, the energy E_v of the oscillator is given by equation 1. The derivation of this equation is given by Herzberg (3).

$$E_v = (v + \tfrac{1}{2})hc\,\omega_e \qquad (1)$$

where v is the vibrational quantum number which may be zero or an integer, c is the velocity of light and ω_e in reciprocal centimeters is the quilibrium frequency of vibration of the oscillator. In general, atomic vibrations are not harmonic, and at least one additional term is required to correct for the anharmonic character of the vibration:

$$E_v = (v + \tfrac{1}{2})hc\,\omega_e - (v + \tfrac{1}{2})^2 xhc\,\omega_e, \qquad (2)$$

where x is an "anharmonicity constant".

For harmonic oscillators transitions are restricted to changes of unity in the quantum number v. For anharmonic oscillations new selection rules permit other transitions but with a smaller degree of probability. When the vibrational energy of a molecule changes from the initial level where v is 0 to that in which v is 1 energy must be absorbed by the molecule in an amount,

$$E = E_1 - E_0 = (1 - 2x)hc\,\omega_e. \qquad (3)$$

The frequency equivalent of the energy E is given by

$$\omega = E/hc \quad cm^{-1} \qquad (4)$$

Therefore, if a quantum of light of frequency ω strikes the molecule, it will be absorbed by the molecule leading to the transi-

tion defined by equation 3. This absorption gives rise to a fundamental band in the molecular spectrum. When the initial value of v is 0 and the final value is 2, the energy change $(1 - 3x)2hc\omega_e$, the frequency of the spectral band is $(1 - 3x)2\omega_e$, and is known as the first overtone. Similarly, expressions can be derived for higher overtones but the probability of their occurance is rather small. If a heterochromatic beam of light with frequencies corresponding to the infrared region is passed through an assemblage of the molecules and the intensity of the transmitted radiation is plotted as a function of wavelength or frequency, the infrared spectrum of the molecule is obtained. Bands will appear in the spectrum at the frequencies corresponding to the fundamental and overtones of the various atomic vibrations within the molecule. Experiments have demonstrated that certain atomic groups give rise to absorption of a specific nature, irrespective of the molecule in which they appear. Therefore, an infrared spectrum offers direct information concerning the atomic linkages present. Since no two compounds have identical structures, their infrared spectra must differ, giving a unique "fingerprint" of the molecule. Not all molecules exhibit infrared absorption. Radiation may be absorbed or emitted only by a system whose electrical configuration is changing relative to a point in space, meaning that a vibration can result in absorption only if the electric moment of the molecule changes in the course of that vibration. Consequently, homopolar diatomic molecules such as H_2 and O_2 do not absorb in the infrared.

Light absorption in other regions of the electromagnetic

spectrum results from other types of intramolecular motion. Transitions in the energy levels of the electrons are produced by the vibrational transitions. Absorptions of this type arise in the visible or ultravoilet regions. Changes in molecular rotation result in absorptions in the far infrared region. The various regions are not sharply defined and there is considerable overlapping.

Analysis of infrared spectra is complicated by the superposition of vibrational and rotational absorption, symmetry factors, and molecular complexity. Since nonlinear molecules possess $3n-6$ normal modes of vibration, where n is the number of atoms in the molecule, a mathematical calculation of frequencies is impracticable for larger molecules. An empirical approach to infrared spectral analysis has met with considerable success.

As mentioned previously, it has been observed that certain chemical bonds or atomic groups give rise to characteristic frequencies irrespective of the molecule in which they appear. Such observations have led to the introduction of the concept of valence and deformation vibrations. For nearly every bond there is a vibration in which the bond is stretched and one of smaller frequency in which the bond is bent.

The occurrence of characteristic frequencies is best explained by a consideration of the C-H, O-H, and N-H vibrations. The mass of the hydrogen nucleus is much smaller than the other nuclei, therefore its vibration will be much greater. To a first approximation the hydrogen nucleus may be considered to be vibrating against an infinitely large mass. In such a case the vibration

frequency depends only on the force by which the hydrogen is bound
to the remainder of the molecule. An approximate expression for
the vibration frequency is

$$\omega_e = 1/2\pi c \ (k/m_H)^{\frac{1}{2}} , \qquad (5)$$

where m_H is the mass of the hydrogen and k is the force constant
of the bond between the two nuclei. Since hydrogen is always an
end atom it can vibrate in the line of the bond or perpendicular
to the bond. Each mode of vibration will have a unique value for
k. Corresponding characteristic frequencies are observed for the
two modes of vibration. The characteristic frequency of a partic-
ular mode is found to be of very nearly the same magnitude irrespec-
tive of whether the hydrogen is bonded to carbon, oxygen, or nitro-
gen. The bond-stretching vibrations for the O-H, C-H, and N-H bonds
all occur in the 3 micron region.

These considerations can only be applied to bonds involving
end atoms whose mass is small compared to the other atoms. Exper-
iments have shown, however, that bonds involving two heavier atoms
have characteristic frequencies even if none are end atoms pro-
vided that the force constants of the different bonds are differ-
ent when the masses are of a similar magnitude. Thus for a mole-
cule containing the group, $-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-O-$ or $-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}-N\overset{\diagup}{\diagdown}$, each bond
would not exhibit a characteristic frequency since both the force
constants and masses are similar; however, for the groups, $-\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}=O$
or $-\overset{|}{\underset{|}{C}}-C\equiv N-$, there would be characteristic frequencies for each
of $C-C$, $C=O$, and $C\equiv N$.

These are general considerations assuming only forces between

adjacent atoms. If a particular bond is present in two molecules, the absorption frequency will be the same in both cases only if the other portions of the two molecules are electronically similar. This is due to the influence exerted on the bond force constant by the surroundings. Such things as bond angles, hydrogen bonding, unsaturation, conjugation, and other resonance effects have been shown to influence the absorption properties of various atomic groups. If there is a possible resonance structure for which the contribution is large, there often appears a splitting of the absorption band. This is due to a slight change in the value of the force constant, which in turn produces the corresponding shift in the frequency.

Some of the frequencies observed may thus be theoretically related to certain groups within the molecule. In practice this assignment of frequencies is usually accomplished empirically. The empirical method is somewhat less certain but does yield good indications of molecular structure.

A classification of the known bands and their approximate positions is given by Randall, et al. (16). Carbonyl groups produce absorptions in the range from 5.45 to 6.5 microns and are the most stable in position. They are the strongest bands in that region. The $C \equiv N$ bond produces bands in the general region of 6 microns. Vibrations of the $C \equiv C$ bond usually result in such a small change in dipole moment that the intensity of the absorption is very low. The vibrations are always found on the long wavelength side of 6 microns. Since the appearance of most bands in the range of 5 to 7 microns are due to the vibration of doubly

bonded atoms, this region has come to be known as the "double bond" region. In general, bands appearing beyond 7 microns are due to skeletal vibrations of the molecule as a whole. These bands give rise to the unique character of a given molecular spectrum. This region is useful in identifying molecules of close resemblance. Compilations of reference spectra to facilitate compound identification have been made by Barnes et al. (4), Randall et al. (16), and the American Petroleum Institute Catalogue of Infrared Spectra of Hydrocarbons.

EXPERIMENTAL PROCEDURE

Infrared Techniques

The instrument used in this work was the Perkin-Elmer Infrared Spectrometer Model 12A, equipped with a gear-driven, automatic wavelength drive coupled with a micrometer slit width control. A standard sodium chloride prism was used in the range of 2 to 14 microns. The transmitted radiation was detected by a vacuum thermocouple, amplified by a General Motors breaker amplifier, and recorded by a Leeds and Northrup type G recorder.

Spectra studies in this investigation were obtained for all substances in the solid state by depositing an appropriate solution on a supporting plate and evaporating in a dessicator over phosphorus pentoxide. The plates were of fused silver chloride and had the dimensions: 30 millimeters x 30 millimeters x 1.05 millimeters. Silver chloride was chosen for two reasons: (1) this material was found to transmit over 80 percent of the incident ra-

diation throughout the spectral region studied; and (2) the water
insolubility of silver chloride made possible the use of aqueous
solutions. The supporting plate was placed in the light path at
the position designated as C in Fig. 1, and the spectrum over the
desired wavelength range was obtained by the common cell in - cell
out method.

Although the thin film technique described here is commonly
employed in infrared studies of proteins as well as other organic
substances (16), its use in quantitative work is limited since
slight variations in film thickness produce sharp changes in the
amount of transmitted radiation. Attempts to adapt this method
to quantitative use have met with little success. Such an attempt
was made during the course of this investigation. Equal volumes
of solutions of varying concentrations were deposited upon sup-
porting plates fitted with rubber perimeters of equal inside
dimensions. Only plates showing a variance of less than 5 per-
cent transmission of radiation were used. It was believed that
the thickness of the film thus obtained would be a function of
the concentration of the original solution, and a plot of optical
density against concentration would result in a straight line. In
general, a straight line tendency was noted but the deviations of
many of the individual points made the calibration unreliable.

Another method used in this investigation for obtaining
infrared spectra was the well-known mineral oil mull method. The
solid sample was suspended in mineral oil and the suspension
placed between two sodium chloride plates separated by a metal
spacer of known thickness. The spectra obtained in this manner
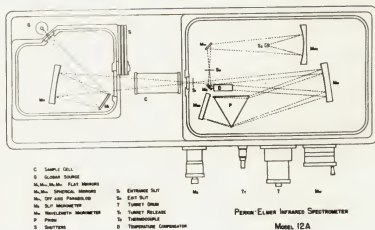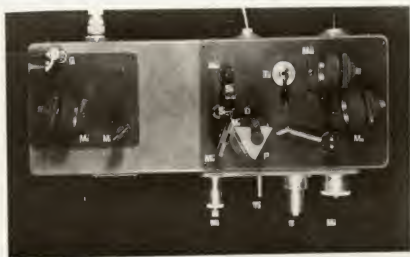
C   SAMPLE CELL
G   GLOBAR SOURCE
M₁,M₂,M₃,M₄   FLAT MIRRORS
M₅,M₆   SPHERICAL MIRRORS
M₇   OFF AXIS PARABOLOID
M₈   SLIT MICROMETER
M₉   WAVELENGTH DRUM
P   PRISM
S   SHUTTERS

S₁   ENTRANCE SLIT
S₂   EXIT SLIT
T   TURRET DRUM
T₁   TURRET RELEASE
T₂   THERMOCOUPLE
D   TEMPERATURE COMPENSATION

PERKIN-ELMER INFRARED SPECTROMETER
MODEL 12A

Fig. 1.   The Perkin-Elmer Infrared Spectrometer Model 12A.

were found to be unsatisfactory due to the radiation scattering effects of the suspended particles. It was found that decreasing the particle size by grinding gave more satisfactory spectra; however, the possibility of protein denaturation prohibited the grinding of that substance. In addition to scattering effects, two other disadvantages encountered in this method caused its discontinuation. The particles were found to settle out of the suspension, thus causing changes in concentration and consequently altering the optical density of the absorption bands. Also, the absorption of the mineral oil masked important absorption bands of the sample.

The infrared spectra included in this thesis are characteristic ones, chosen from several runs of each substance. The spectra were chosen from films of approximately the same thickness as determined from the heights of the characteristic absorption bands.

Other Experimental Methods

The spectrum of the thermal decomposition product of dry histidine was obtained by heating a thin film of the amino acid. The film had previously been deposited on a silver chloride from an aqueous solution. The film was heated at approximately 175 degrees Centigrade for 24 hours in a drying oven. After heating, the plate was allowed to cool to room temperature before obtaining the spectrum. In addition, a sample of histidine was refluxed in concentrated hydrochloric acid solution for 10 minutes. A

sample of the reaction mixture was then allowed to evaporate from
a silver chloride plate and the spectrum of the resulting film was
then obtained.

In the study of the interaction of histamine with bovine serum
albumin, a 2 percent solution of the protein in distilled water
was prepared. Three milliliter portions of the solution were
placed in cellophane dialysis bags to which had been added enough
histamine or urea to make a 6 molar solution. Each dialysis bag
was then placed in 10 milliliters of distilled water. The system
was allowed to stand at 4 degrees Centigrade for 120 hours. The
water surrounding the dialysis bags was changed at 8 hour inter-
vals. Upon completion of the dialyzing period the infrared spectra
of the solutions within the dialysis bags were obtained by the
method previously described. The spectrum of heat denatured pro-
tein was obtained by heating a film of the native protein for 1
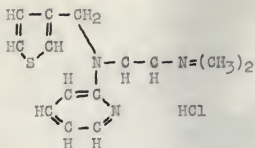hour at 70 degrees.

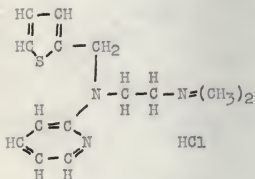## RESULTS AND DISCUSSION

Antihistamine Spectra

The infrared spectra of three antihistamines are given in
Fig. 2. The names and structural formulas of these compounds are
given below.
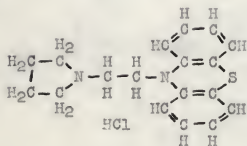
Pyrrolazoate HCl

Thenfadil HCl

Thenylpyramine HCl

These compounds were used in learning the infrared technique. They have been included in this thesis as reference spectra for work done in the future with these, or similar, compounds.

While no detailed analysis of the spectra has been attempted, Table 1 gives the principal absorption bands of the three compounds and, in some cases, possible band assignments. The strong band at 6.26 microns appearing in the spectra of the latter two compounds and the doublet at 6.26 and 6.34 microns in the spectrum of the first compound are due probably to the vibrations of the sulfur-containing rings. The strong band at approximately 6.90 microns in the latter two spectra has been assigned to the pyridine ring vibrations. The bands at approximately 2.94, 3.40, 3.85 and 4.04 microns common to all three spectra have been tentatively assigned to the N-H stretching, CH₂ stretching, and HCl vibrations, respectively. The appearance of a strong, broad band in the 13
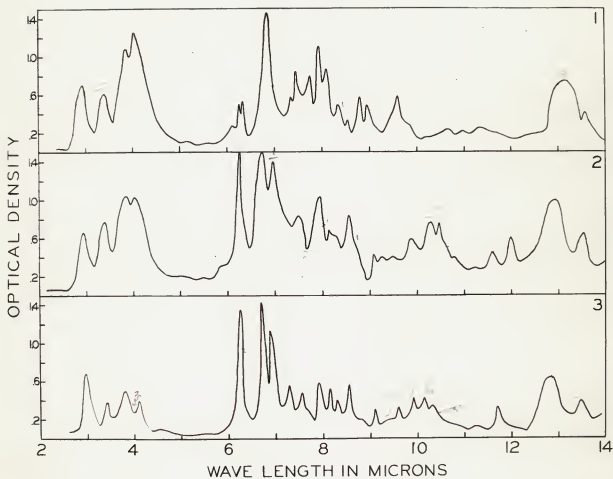
Fig. 2.   The infrared spectra of three antihistamines.   (1) Pyr-
          rolazoate HCl. (2) Thenfadil HCl. (3) Thenylpyramine HCl.

micron region with a band of lesser intensity at about 13.5 microns
has led to a tentative assignment to the vibrations of the ethylene
diamine structure since this is the structure common to all three
compounds. Such skeletal vibrations generally produce bands in
this region.

Table 1. Principle absorption frequencies in microns of three
antihistamines with some probable band assignments. The
amount of absorption is indicated as very strong (VS);
strong (S); medium (M); and weak (W). The mode of vibra-
tion is indicated as stretching (s); bending (b); and
ring (r).

Pyrrolazoate HCl		Thenfadil HCl		Thenylpyramine HCl	
2.94 M	N-H s	2.94 M	N-H s	2.97 M	N-H s
3.40 M	CH$_2$ s	3.40 M	CH$_2$ s	3.44 W	CH$_2$ s
3.85 S	HCl	3.85 S	HCl	3.82 M	HCl
4.04 S	HCl	4.04 S	HCl	4.10 W	HCl
6.26 M	r	6.26 VS	r	6.26 VS	r
6.34 M	r				
		6.74 VS C C r		6.70 VS C C r	
6.85 VS C C r					
		6.96 S	r	6.87 S	r
				7.28 M	
7.45 S	C-H b	7.50 M C-H b		7.55 W	C-H b
7.75 S					
7.94 S	C-H b	7.96 S C-H b		7.92 M	C-H b
8.10 S		8.14 W		8.14 W	
8.33 W				8.29 W	
8.54 W	C-H b	8.55 M C-H b		8.55 M	C-H b
8.80 M	r				
8.95 M	r	9.08 W	r	9.08 W	r
		9.25 W			
9.60 M		9.48 W		9.60 W	
		10.28 S		10.14 W	
10.64 W		10.64 S		10.32 W	
10.96 W		10.78 W			
11.34 W		11.24 W		11.24 W	
		11.58 W			
		11.98 M			
13.14 S N-C-C-N		12.94 S N-C-C-N		12.84 S N-C-C-N	
13.58 M "		13.54 M "		13.48 M "	

The Spectra of Histidine Monohydrochloride and Histamine

The infrared spectra of the amino acid monohydrochlorides have been studied extensively. A general discussion of these spectra is given by Randall et al. (16). The spectrum of histidine monohydrochloride was obtained by Heintz (7) in 1937. The spectrum obtained in this investigation was found to be in closer agreement with the general amino acid spectra than that published by Heintz. This is probably due to the poor resolution of the spectrometer used by that investigator.

Amino acid spectra are characterized by several distinct absorption bands: (1) a series of low-intensity bands in the region from 3.45 to 4.0 microns; (2) a band of moderate absorption near 5.0 microns; (3) a strong band near 6.20 microns generally assigned to the vibration of the carboxylate ion; (4) a weaker band occurring near 6.15 microns usually referred to as Amino Acid I; and (5) the Amino Acid II band appearing between 6.47 and 6.66 microns. In the spectrum of histidine, as shown in Fig. 3, an additional band is found at 2.98 microns. This can be assigned to the N-H stretching vibrations of the amine group. The Amino Acid I absorption apparently is masked by that of the carboxylate ion.

No reference could be found in the literature pertaining to the infrared spectrum of histamine. The spectrum of this compound as shown in Fig. 3 seems to have the characteristic aliphatic amine absorptions. The strong bands at 3.22 and 6.40 microns are due to N-H stretching and deformation vibrations, respectively. The bands at 3.48 and 6.70 are due to C-H stretching and bending vibra-

tions. The appearance of a moderately strong band at about 7.5 microns in both histidine and histamine spectra might indicate that it is due to the vibrations of the imidazole ring.

Fig. 3 gives a comparison of the spectra of the thermal decomposition product of dry histidine and of the reaction mixture after refluxing a concentrated hydrochloric acid solution of histidine with the spectra of histidine and histamine. In the spectrum of the decomposition product of dry histidine partial deamination is indicated since the intensity of the bands assigned to the amine vibrations has been greatly decreased. The intensity of the carboxyl band is not changed greatly but the band has been shifted slightly to a shorter wavelength. This may be attributed to the release of the carboxyl group from the carboxylate ion form (16) after the amino acid has been deaminated.

Gross differences are found in the spectrum of the acid-refluxed reaction mixture. In this case the carboxyl band has been shifted from 6.20 microns to 5.74 microns. This new position is a characteristic one for ester or ketone structures. The possibility of esterification is strengthened by the appearance of a strong band at 8.14 microns which might be due to C-O-C vibrations. It was not possible to determine whether the imidazole ring had been ruptured. The retention of the C-N band at 6.70 microns suggests that the ring is intact. It is apparent from an examination of the spectra of Fig. 3 that histamine is not a major product of this reaction.
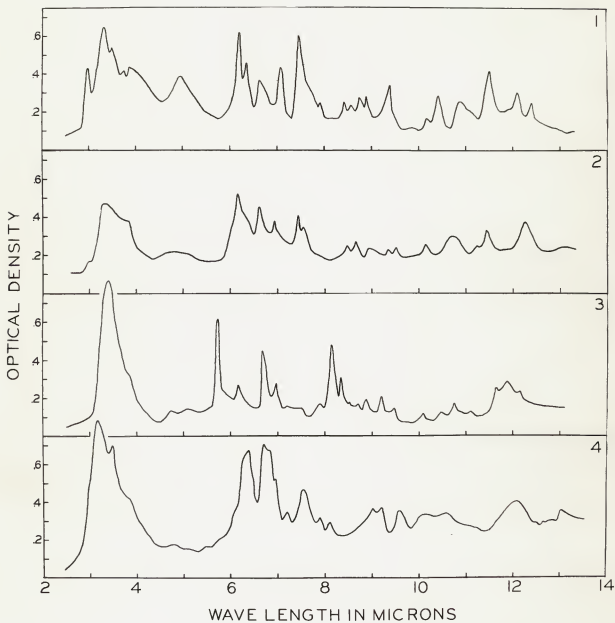
Fig. 3. Infrared absorption spectra. (1) Histidine monohydro-
chloride. (2) Thermal decomposition product of dry
histidine. (3) Acid refluxed reaction mixture. (4) His-
tamine.

The Effect of Histamine upon Bovine Serum Albumin

The infrared absorption of proteins has been studied quite extensively (5,7,12). In this investigation the spectrum of bovine serum albumin was obtained over the wavelength range of 2 to 10 microns. It was found to be in very close agreement with that published by Klotz, Griswald, and Gruen (12).

The effect produced upon the protein by the presence of histamine was studied by noting the change in the spectrum of the protein. Only one change was noted. A new band was found to appear at 3.22 microns. The characteristic absorption of histamine was found nowhere throughout the region studied. Buswell, Krebs, and Rodebush (5), in their investigation of the infrared spectra of sixteen proteins, have found that the appearance of a new absorption band at 3.22 microns accompanies what they believe to be denaturation of the protein. Protein denaturation is considered here to be any non-proteolytic modification of the structure of a native protein producing definite changes in its chemical or physical properties (15). Denaturation may be brought about by physical or mechanical means such as grinding or heating, or by chemical action of certain substances. Urea in 6 molar concentration is reported to denature serum albumin (15).

In order to determine if the histamine action upon the protein was similar to that produced by a common denaturing agent, urea was substituted for histamine in the experiment. In addition, the spectrum of heat denatured protein was obtained for comparison. Fig. 4 gives a graphical comparison of the results
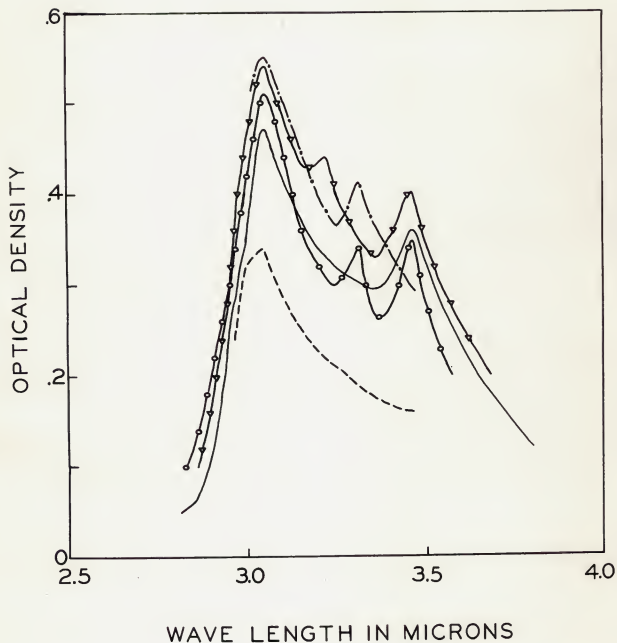
Fig. 4. The infrared absorption spectra of bovine serum albumin in the 3 micron region. —— native protein; .——. old protein; o——o heat denatured protein; ---- urea denatured protein; ▼——▼ protein after interaction with histamine.

obtained in this experiment. Histamine produces the new band at
3.22 microns with no change in the characteristic absorptions at
3.05 and 3.46 microns. In heat denaturation, the new band appears
3.31 microns with no change in the characteristic absorptions.
Urea produces a slight change in the shape of the 3.05 micron band
and apparently shifts the 3.46 micron band toward the shorter wave-
lengths.

The spectrum of a sample of bovine serum albumin which, after
long periods of exposure to room temperature, was believed to be
at least partially denatured, is included in Fig. 4. This sample
is referred to hereafter as old protein. The spectrum of the old
protein shows a shift of the absorption band from 3.46 microns to
3.31 microns. This is interesting in view of the fact that heat
denaturation produces a band at this position.

These observations may be interpreted from either of two
viewpoints. First, the new bands appearing in the spectrum may
be considered to be due to hydrogen bonding of the type N-H···O,
in which case the band would result from a shift from the 3.05
micron position. This band is due to the N-H stretching vibra-
tion. Second, the new bands might be due to some change in the
molecular configuration effecting the C-H vibrations and causing
a shift from the normal C-H stretching band at 3.46 microns to
shorter wavelengths. The unfolding of the protein molecule which
supposedly accompanies denaturation might produce such an effect.
The extent to which the band is shifted might then give an in-
dication of the degree of denaturation. If such is the case,
urea apparently is the most severe denaturing agent.

Buswell, Krebs, and Rodebush give the first interpretation
to their observations. They state:

> In nature one might expect that in the presence of ex-
> cess water it would bond to half the hydrophilic groups, thus

$$R - N - C -$$
$$H_2CH \quad \parallel$$
$$\qquad O$$
$$O \quad \vdots$$
$$\parallel \quad H$$
$$-C - N - R$$
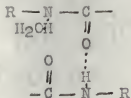
> preventing ring formation. This water might be considered
> to be 'water of constitution'. ...on severe dehydration
> the water of constitution would be irreversibly removed and
> the ring structure,

$$R - N - C -$$
$$H \quad \parallel$$
$$\vdots \quad O$$
$$O \quad \vdots$$
$$\parallel \quad H$$
$$-C - N - R \quad ,$$
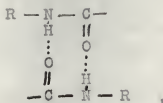
which is very stable, would be formed.

By analogy it might seem reasonable to suppose that the band pro-
duced in the spectrum of the protein by the action of histamine
is due to the N-H···O bond in the ring dimer structure. If this
is true, the action of histamine must in some way cause the pro-
tein to lose its water of constitution. It seems apparent from
these data that histamine is not irreversibly bound by the protein.
Studies of this problem being carried out using other methods seem
to indicate that histamine is reversibly bound by the protein. A
better understanding of protein denaturation is required before
the spectral data of Fig. 4 can be given a positive interpretation.

EXTENSIONS

During the course of this work several interesting problems have arisen concerning the chemical behavior of histamine and histidine. A few observations which might warrant further study are given below.

Some indications were found that histamine forms complexes in aqueous solutions of some heavy metal ions. The identification of histamine in the presence of histidine and other substances has presented a difficult problem in the past. Complex formation might lead to a simple spectrophotometric method for histamine determination.

Further studies of the conversion of histidine to histamine are needed to fully understand the mechanism whereby this reaction takes place in the body. More data is needed regarding the infrared absorption of the imidazole ring in order to determine whether the ring is ruptured under the experimental conditions used.

It is believed that the methods of infrared spectroscopy offer a convenient method for studying the problem of protein denaturation. Improvement of the film technique commonly employed in determining the infrared spectra of proteins is needed.

SUMMARY

The infrared spectra of three antihistamines have been obtained. An attempt has been made to assign the major absorption bands to particular intramolecular vibrations.

The infrared spectra of histidine monohydrochloride and histamine have been obtained and are compared. Band assignments have been made where possible. In addition, the spectra of the decomposition products obtained by heating dry histidine and by heating a concentrated hydrochloric acid solution of histidine have been included. It is evident from these spectra that histamine is not a major product in these reactions. Evidence indicates that dry histidine is deaminated by the action of heat.

The effect of histamine on bovine serum albumin has been studied. Changes were noted in the spectra of the protein after interaction with histamine. A new band was found to appear at 3.22 microns. This is somewhat similar to results obtained by denaturing the protein. It is apparent that histamine in some way alters the configuration of the protein molecule. Histamine is not irreversibly bound by the protein.

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INFRARED STUDIES OF HISTAMINE, HISTIDINE,
AND SEVERAL ANTIHISTAMINES

by

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The methods of infrared spectroscopy have been employed in studying the reported conversion of histidine to histamine by thermal decomposition. Also, the interaction of histamine with bovine serum albumin was studied by noting the change produced in the protein spectrum. This change was compared with that produced by denaturing the protein by urea and by heat. Certain similarities were noted. The spectra of three antihistaminic compounds were obtained and compared. An attempt was made to assign vibrational modes to the major absorption frequencies.

The instrument used in this work was the Perkin-Elmer Infrared Spectrometer Model 12A, equipped with a gear-driven, automatic wavelength drive coupled with a micrometer slit width control. A standard sodium chloride prism was used in the range 2 to 14 microns. The transmitted radiation was detected by a vacuum thermocouple, amplified by a General Motors breaker amplifier, and recorded by Leeds and Northup type G recorder.

Spectra were obtained for all substances in the solid state by depositing an appropriate solution on a supporting plate and evaporating in a dessicator over phosphorus pentoxide. The supporting plates were of fused silver chloride. This matterial was found to transmit over 80 percent of the incident radiation throughout the spectral range studies. In addition, the water insolubility of silver chloride made possible the use of aqueous solutions.

An attempt was made to adapt this technique to quantitative determinations. Equal volumes of solutions of varying concentra-

tion were deposited upon supporting plates fitted with rubber
perimeters of equal inside dimensions. Only plates showing a
variance of less than 5 percent transmission of radiation were
used. The thickness of the film should be a function of the
concentration of the original solution and a plot of optical
density against concentration should yield a straight line.
While a straight line tendency was noted, the deviations of
many of the individual points made the calibration unreliable.

A comparison of the spectra of histidine and histamine
with those of the thermal decomposition product of dry histidine
and the reaction mixture after refluxing a concentrated hydro-
chloric acid solution of histidine indicates that histamine is
not a major product in these reactions. Apparently the dry
histidine is deaminated by intensive heating. Esterification
seems to be indicated in the case of the refluxed acid solution.

In studying the effect of histamine on bovine serum
albumin a 2 percent solution of the protein was placed in a
cellophane dialysis bag containing enough histamine to make a
6 molar solution. The bag was placed in 10 milliliters of
distilled water. The system was allowed to stand at 4 degrees
Centigrade for 120 hours. The water surrounding the dialysis
bag was changed at 8 hour intervals. After this period the
infrared spectrum of the solution inside the bag was obtained.
A new absorption band was found to appear at 3.22 microns. This
corresponds to the results obtained by other workers upon
denaturation of various proteins. Urea was substituted for
histamine in the experiment and, in addition, the spectrum of

heat denatured protein was obtained by heating a film of
native protein for 1 hour at 70 degrees. In heat denaturation,
the new band appears at 3.31 microns. Urea apparently produces
a shift of the C-H vibration at 3.46 microns. An attempt is
made to explain the spectral changes on the basis of the for-
mation of the N-H...O bond, or as the result of changes in
molecular configuration which produces shifts in the C-H
vibration normally found at 3.46 microns.